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(54) Title: GLUTAMATE RECEPTOR

(57) Abstract

The present invention relates to a human metabotropic glutamate receptor (hmGluR) subtype, isolated nucleic acids coding therefor, host cells producing a protein of the invention, methods for the preparation of such protein, nucleic acids and host cells, and uses thereof. Furthermore, the invention provides antibodies directed against a hmGluR protein of the invention.

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### Glutamate Receptor

The present invention relates to a human metabotropic glutamate receptor (hmGluR) subtype, isolated nucleic acids coding therefor, host cells producing a protein of the invention, methods for the preparation of such protein, nucleic acids and host cells, and uses thereof. Furthermore, the invention provides antibodies directed against a hmGluR protein of the invention.

Metabotropic glutamate receptors (hmGluR) belong to the class of G-protein (guanine nucleotide binding protein) coupled receptors which upon binding of a glutamatergic ligand may transduce an extracellular signal via an intracellular second messenger system such as calcium ions, a cyclic nucleotide, diacylglycerol, inositol 1,4,5-triphosphate into a physiological response. Possessing seven putative transmembrane spanning segments, preceded by a large extracellular amino-terminal domain and followed by a large carboxy-terminal domain metabotropic glutamate receptors are characterized by a common structure. Based on the degree of sequence identity at the amino acid level the class of mGluR can be divided into different subfamilies comprising individual receptor subtypes (Nakanishi, Science 258, 597-603 (1992)). Each mGluR subtype is encoded by a unique gene. Regarding the homology of an individual mGluR subtype to another subtype of a different subfamily, the amino acid sequences are less than about 50 % identical. Within a subfamily the degree of sequence identity is generally less than about 70 %. Thus a particular subtype may be characterized by its amino acid sequence homology to another mGluR subtype, especially a subtype of the same mammalian species. Furthermore, a particular subtype may be characterized by its region and tissue distribution, its cellular and subcellular expression pattern or by its distinct physiological profile, e.g. by its electrophysiological and pharmacological properties.

The amino acid L-glutamate being the major excitatory neurotransmitter, glutamatergic systems are presumed to play an important role in numerous neuronal processes including fast excitatory synaptic transmission, regulation of neurotransmitter releases, long-term potentiation, learning and memory, developmental synaptic plasticity, hypoxic-ischemic damage and neuronal cell death, epileptiform seizures, as well as the pathogenesis of several neurodegenerative disorders. Up to today, no information is available on human metabotropic glutamate receptor (hmGluR) subtype 2, e.g. on the amino acid sequence or tissue distribution. This lack of knowledge particularly hampers the search for human therapeutic agents capable of specifically influencing any disorder attributable to a defect

in the glutamatergic system. In view of the potential physiological and pathological significance of metabotropic glutamate receptors, there is a need for human receptor subtypes and cells producing such subtypes in amounts sufficient for elucidating the electrophysiological and pharmacological properties of these proteins. For example, drug screening assays require a purified human receptor protein in an active form, which has not yet been attainable.

It is an object of the present invention to fulfill this need, namely to provide hmGluR subtype 2, a nucleic acid coding therefor and host cells producing such subtype. HmGluR2 is potently activated by (2S,3S,4S)- $\alpha$ -(carboxycyclopropyl)-glycine (L-CCG-I) and, when expressed e.g. in Chinese hamster ovary (CHO) cells or baby hamster kidney (BHK) cells, negatively coupled to adenylate cyclase via G protein. Using a system comprising a recombinant hmGluR subtype of the invention in screening for hmGluR reactive drugs offers (among others) the possibilities of attaining a greater number of receptors per cell giving greater yield of reagent and a higher signal to noise ratio in assays as well as increased receptor subtype specificity (potentially resulting in greater biological and disease specificity).

More specifically, the present invention relates to hmGluR2 having the amino acid sequence depicted in SEQ ID NO:2.

According to the invention the expression "hmGluR subtype" refers to a purified protein which belongs to the class of G protein-coupled receptors and which upon binding of a glutamatergic ligand transduces an extracellular signal via an intracellular second messenger system. In such case, the subtype of the invention is characterized in that it modifies the level of a cyclic nucleotide (cAMP, cGMP). Alternatively, signal transduction may occur via direct interaction of the G protein coupled to the receptor subtype of the invention with another membrane protein, such as an ion channels. HmGluR2 is believed to be encoded by a distinct gene which does not encode another metabotropic glutamate receptor subtype. A particular subtype may be characterized by its distinct physiological profile, preferably by its signal transduction and pharmacological properties. Pharmacological properties are e.g. the selectivity for agonists and antagonist responses.

As defined herein, a glutamatergic ligand is e.g. L-glutamate or another compound interacting with, and particularly binding to, a hmGluR subtype in a glutamate like

manner, such as ACPD (1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid), an ACPD-like ligand, e.g. QUIS (quisqualate), L-2-amino-4-phosphobutyric acid (AP4), L-CCG-I, and the like. Other ligands, e.g. (R,S)- $\alpha$ -methyl-4-carboxyphenylglycine (MCPG) or  $\alpha$ -methyl-L-AP4, may interact with the receptor of the invention in such a way that binding of a glutamatergic ligand is prevented.

As used hereinbefore or hereinafter, the terms "purified" or "isolated" are intended to refer to a molecule of the invention in an enriched or pure form obtainable from a natural source or by means of genetic engineering. The purified protein, DNA and RNA of the invention may be useful in ways that the protein, DNA and RNA as they naturally occur are not, such as identification of compounds selectively modulating the expression or the activity of the hmGluR of the invention.

Purified hmGluR of the invention means hmGluR2 which has been identified and is free of one or more components of its natural environment. Purified hmGluR includes purified hmGluR of the invention in recombinant cell culture. The enriched form of the subtype refers to a preparation containing said subtype in a concentration higher than natural, e.g. a cellular membrane fraction comprising said subtype. If the subtype is in a pure form it is substantially free from other macromolecules, particularly from naturally occurring proteinaceous contaminations. If desired, the subtype may be solubilized. A preferred purified hmGluR2 of the invention is a recombinant protein. Preferably, the subtype of the invention is in an active state meaning that it has both ligand binding and signal transduction activity. Receptor activity is measured according to methods known in the art, e.g. using a binding assay or a functional assay, e.g. an assay as described below.

The invention is further intended to include variants of the receptor subtype of the invention. For example, a variant of the hmGluR subtype of the invention is a functional or immunological equivalent of said subtype. A functional equivalent is a human protein displaying a physiological profile essentially identical to the profile characteristic of the hmGluR2 having the amino acid sequence set forth in SEQ ID NO:2. Furthermore, a functional equivalent has more than 70 %, preferably more than 90 %, sequence identity with the protein having the amino acid sequence set forth in SEQ ID NO:2. Accordingly, a functional equivalent does not include another hmGluR subtype of the same subfamily, e.g. hmGluR3. The physiological profile in vitro and in vivo includes receptor effector function, electrophysiological and pharmacological properties, e.g. selective interaction with agonists or antagonists. Exemplary functional equivalents may be splice variants

encoded by mRNA generated by alternative splicing of a primary transcript, amino acid mutants and glycosylation variants. An immunological equivalent of the hmGluR2 having the amino acid sequence set forth in SEQ ID NO:2 is a protein or peptide capable of generating antibodies specific for said subtype. Portions of the extracellular domain of the receptor, e.g. peptides consisting of at least 6 to 8 amino acids, particularly about 20 amino acids, are considered particularly useful immunological equivalents.

Further variants included herein are membrane-bound and soluble fragments and covalent or aggregative conjugates with other chemical moieties, these variants displaying one or more receptor functions, such as ligand binding or signal transduction. The fragments of the invention are obtainable from a natural source, by chemical synthesis or by recombinant techniques. Due to their capability of competing with the endogenous counterpart of the hmGluR subtype of the invention for its endogenous ligand, fragments, or derivatives thereof, comprising the ligand binding domain are envisaged as therapeutic agents.

Covalent derivatives include for example aliphatic esters or amides of a receptor carboxyl group, O-acyl derivatives of hydroxyl group containing residues and N-acyl derivatives of amino group containing residues. Such derivatives can be prepared by linkage of functionalities to reactable groups which are found in the side chains and at the N- and C-terminus of the receptor protein. The protein of the invention can also be labeled with a detectable group, for example radiolabeled, covalently bound to rare earth chelates or conjugated to a fluorescent moiety.

Further derivatives are covalent conjugates of a protein of the invention with another protein or peptide (fusion proteins). Examples are fusion proteins comprising different portions of different glutamate receptors. Such fusion proteins may be useful for changing the coupling to G-proteins and/or improving the sensitivity of a functional assay. For example, in such fusion proteins or chimeric receptors, the intracellular domains of the subtype of the invention may be replaced with the corresponding domains of another mGluR subtype, particularly a hmGluR subtype, e.g. a hmGluR subtype belonging to another subfamily. Particularly suitable for the construction of such a chimeric receptor are the intracellular domains of a receptor which activates the phospholipase C/Ca<sup>2+</sup> signaling pathway, e.g. mGluR1 (Masu et al., Nature 349, 760-765) or mGluR5. An intracellular domain suitable for such an exchange is e.g. the second intracellular loop, also referred to as i2 (Pin et al., EMBO J. 13, 342-348 (1994)). Thus it is possible e.g. to

analyze the interaction of a test compound with a ligand binding domain of a receptor of the invention using an assay for calcium ions. The chimeric receptor according to the invention can be synthesized by recombinant techniques or agents known in the art as being suitable for crosslinking proteins.

Aggregative derivatives are e.g. adsorption complexes with cell membranes.

In another embodiment, the present invention relates to a composition of matter comprising the hmGluR subtype of the invention.

The proteins of the invention are useful e.g. as immunogens, in drug screening assays, as reagents for immunoassays and in purification methods, such as for affinity purification of a binding ligand.

A protein of the invention is obtainable from a natural source, e.g. by isolation from brain tissue, by chemical synthesis or by recombinant techniques.

The invention further provides a method for preparing the hmGluR subtype of the invention, said method being characterized in that suitable host cells producing the receptor subtype of the invention are multiplied in vitro or in vivo. Preferably, the host cells are transformed (transfected) with a hybrid vector comprising an expression cassette comprising a promoter and a DNA sequence coding for said subtype which DNA is controlled by said promoter. Subsequently, the hmGluR subtype of the invention may be recovered. Recovery comprises e.g. isolating the subtype of the invention from the host cells or isolating the host cells comprising the subtype, e.g. from the culture broth. Particularly preferred is a method for preparation of a functionally active receptor.

HmGluR muteins may be produced from a DNA encoding a hmGluR protein of the invention which DNA has been subjected to in vitro mutagenesis resulting e.g. in an addition, exchange and/or deletion of one or more amino acids. For example, substitutional, deletional and insertional variants of a hmGluR subtype of the invention are prepared by recombinant methods and screened for immuno-crossreactivity with the native forms of the hmGluR.

A protein of the invention may also be derivatized in vitro according to conventional methods known in the art.

Suitable host cells include eukaryotic cells, e.g. animal cells, plant cells and fungi, and prokaryotic cells, such as gram-positive and gram-negative bacteria, e.g. *E. coli*. Preferred eukaryotic host cells are of amphibian or mammalian origin.

As used herein, in vitro means ex vivo, thus including e.g. cell culture and tissue culture conditions.

This invention further covers a nucleic acid (DNA, RNA) comprising a purified, preferably recombinant, nucleic acid (DNA, RNA) coding for the subtype of the invention, or a fragment of such a nucleic acid. In addition to being useful for the production of the above mentioned recombinant hmGluR proteins, these nucleic acids are useful as probes, thus e.g. readily enabling those skilled in the art to identify and/or isolate nucleic acid encoding a hmGluR2 protein of the invention. The nucleic acid may be unlabeled or labeled with a detectable moiety. Furthermore, nucleic acid according to the invention is useful e.g. in a method for determining the presence of hmGluR, said method comprising hybridizing the DNA (or RNA) encoding (or complementary to) hmGluR to test sample nucleic acid and to determine the presence of hmGluR.

Purified hmGluR2 encoding nucleic acid of the invention includes nucleic acid that is free from at least one contaminant nucleic acid with which it is ordinarily associated in the natural source of hmGluR nucleic acid. Purified nucleic acid thus is present in other than in the form or setting in which it is found in nature. However, purified hmGluR2 nucleic acid embraces hmGluR2 nucleic acid in ordinarily hmGluR expressing cells where the nucleic acid is in a chromosomal location different from that of natural cells or is otherwise flanked by a different DNA sequence than that found in nature. The hmGluR2 gene maps to human chromosome 3.

In particular, the invention provides a purified or isolated DNA molecule encoding a hmGluR2 protein of the invention, or a fragment of such DNA. By definition, such a DNA comprises a coding single DNA, a double stranded DNA consisting of said coding DNA and complementary DNA thereto, or this complementary (single stranded) DNA itself. Preferred is a DNA coding for the above captioned preferred hmGluR2, or a fragment thereof. Furthermore, the invention relates to a DNA comprising a DNA coding for the above captioned preferred hmGluR2 subtype, or a fragment thereof.



More specifically, preferred is a DNA coding for hmGluR2 or a portion thereof, particularly a DNA encoding the hmGluR2 having the amino acid sequence set forth in SEQ ID NO:2, e.g. the DNA with the nucleotide sequence set forth in SEQ ID NO:1.

The nucleic acid sequences provided herein may be employed to identify DNAs encoding further hmGluR subtypes. For example, nucleic acid sequences of the invention may be used for identifying DNAs encoding further hmGluR subtypes belonging to the same receptor subfamily. A method for identifying such DNA comprises contacting human DNA with a nucleic acid probe described above and identifying DNA(s) which hybridize to that probe.

Exemplary nucleic acids of the invention can alternatively be characterized as those nucleic acids which encode a hmGluR subtype of the invention and hybridize to the DNA having the sequence set forth in SEQ ID NO.: 1, or a selected portion (fragment) of said DNA. Preferred are such DNA molecules encoding a hmGluR of the invention which hybridize under high-stringency conditions to the above-mentioned DNAs.

Stringency of hybridization refers to conditions under which polynucleic acids hybrids are stable. Such conditions are evident to those of ordinary skill in the field. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature ( $T_m$ ) of the hybrid which decreases approximately 1 to 1.5°C with every 1% decrease in sequence homology. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of higher stringency, followed by washes of varying stringency.

As used herein, high stringency refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 1 M Na<sup>+</sup> at 65-68 °C. High stringency conditions can be provided, for example, by hybridization in an aqueous solution containing 6x SSC, 5x Denhardt's, 1 % SDS (sodium dodecyl sulfate), 0.1 Na<sup>+</sup> pyrophosphate and 0.1 mg/ml denatured salmon sperm DNA as non specific competitor. Following hybridization, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridization temperature in 0.2- 0.1x SSC, 0.1 % SDS.

Moderate stringency refers to conditions equivalent to hybridization in the above described solution but at about 60-62 °C. In that case the final wash is performed at the hybridization temperature in 1x SSC, 0.1 % SDS.

Low stringency refers to conditions equivalent to hybridization in the above described solution at about 50-52°C. In that case, the final wash is performed at the hybridization temperature in 2x SSC, 0.1 % SDS.

It is understood that these conditions may be adapted and duplicated using a variety of buffers, e.g. formamide-based buffers, and temperatures. Denhart's solution and SSC are well known to those of skill in the art as are other suitable hybridization buffers (see, e.g. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (2nd edition), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA, or Ausubel, F. M., et al. (1993) *Current Protocols in Molecular Biology*, Greene and Wiley, USA). Optimal hybridization conditions have to be determined empirically, as the length and the GC content of the probe also play a role.

Given the guidance of the present invention, the nucleic acids of the invention are obtainable according to methods well known in the art. The present invention further relates to a process for the preparation of such nucleic acids.

For example, a DNA of the invention is obtainable by chemical synthesis, by recombinant DNA technology or by polymerase chain reaction (PCR). Preparation by recombinant DNA technology may involve screening a suitable cDNA or genomic library. A suitable method for preparing a DNA of the invention may e.g. comprise the synthesis of a number of oligonucleotides, their amplification by PCR methods, and their splicing to give the desired DNA sequence. Suitable libraries are commercially available, e.g. the libraries employed in the Examples, or can be prepared from neural or neuronal tissue samples, e.g. hippocampus or cerebellum tissue, cell lines and the like.

For an individual hmGluR subtype (and splice variants) the expression pattern in neural or neuronal tissue may vary. Thus, in order to isolate cDNA encoding a particular subtype (or splice variant), it is advantageous to screen libraries prepared from different suitable tissues or cells. As a screening probe, there may be employed a DNA or RNA comprising substantially the entire coding region of hmGluR2, or a suitable oligonucleotide probe based on said DNA. A suitable oligonucleotide probe (for screening involving hybridization) is a single stranded DNA or RNA that has a sequence of nucleotides that includes at least 14 contiguous bases that are the same as (or complementary to) any 14 or more contiguous bases set forth in SEQ ID NO:1. The probe may be labeled with a

suitable chemical moiety for ready detection. The nucleic acid sequences selected as probes should be of sufficient length and sufficiently unambiguous so that false positive results are minimized.

Preferred regions from which to construct probes include 5' and/or 3' coding sequences, sequences predicted to encode ligand binding sites, and the like. For example, either the full-length cDNA clone disclosed herein or fragments thereof can be used as probes. Preferably, nucleic acid probes of the invention are labeled with suitable label means for ready detection upon hybridization. For example, a suitable label means is a radiolabel. The preferred method of labelling a DNA fragment is by incorporating  $^{32}\text{P}$ -labelled  $\alpha$ -dATP with the Klenow fragment of DNA polymerase in a random priming reaction, as is well known in the art. Oligonucleotides are usually end-labeled with  $^{32}\text{P}$ -labeled  $\gamma$ -ATP and polynucleotide kinase. However, other methods (e.g. non-radioactive) may also be used to label the fragment or oligonucleotide, including e.g. enzyme labelling and biotinylation.

After screening the library, e.g. with a portion of DNA including substantially the entire hmGluR2-encoding sequence or a suitable oligonucleotide based on a portion of said DNA, positive clones are identified by detecting a hybridization signal; the identified clones are characterized by restriction enzyme mapping and/or DNA sequence analysis, and then examined, e.g. by comparison with the sequences set forth herein, to ascertain whether they include DNA encoding a complete hmGluR (i.e., if they include translation initiation and termination codons). If the selected clones are incomplete, they may be used to rescreen the same or a different library to obtain overlapping clones. If the library is genomic, then the overlapping clones may include exons and introns. If the library is a cDNA library, then the overlapping clones will include an open reading frame. In both instances, complete clones may be identified by comparison with the DNAs and deduced amino acid sequences provided herein.

Furthermore, in order to detect any abnormality of an endogenous hmGluR2 genetic screening may be carried out using a nucleotide sequence of the invention as hybridization probes. Also, based on the nucleic acid sequences provided herein antisense-type therapeutic agents may be designed.

It is envisaged that the nucleic acid of the invention can be readily modified by nucleotide substitution, nucleotide deletion, nucleotide insertion or inversion of a nucleotide stretch,

and any combination thereof. Such modified sequences can be used to produce a mutant hmGluR subtype which differs from the receptor subtypes found in nature. Mutagenesis may be predetermined (site-specific) or random. A mutation which is not a silent mutation must not place sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins.

The cDNA or genomic DNA encoding native or mutant hmGluR of the invention can be incorporated into vectors for further manipulation. Furthermore, the invention concerns a recombinant DNA which is a hybrid vector comprising at least one of the above mentioned DNAs.

The hybrid vectors of the invention comprise an origin of replication or an autonomously replicating sequence, one or more dominant marker sequences and, optionally, expression control sequences, signal sequences and additional restriction sites.

Preferably, the hybrid vector of the invention comprises an above described nucleic acid insert operably linked to an expression control sequence, in particular those described hereinafter.

Vectors typically perform two functions in collaboration with compatible host cells. One function is to facilitate the cloning of the nucleic acid that encodes the hmGluR subtype of the invention, i.e. to produce usable quantities of the nucleic acid (cloning vectors). The other function is to provide for replication and expression of the gene constructs in a suitable host, either by maintenance as an extrachromosomal element or by integration into the host chromosome (expression vectors). A cloning vector comprises the DNAs as described above, an origin of replication or an autonomously replicating sequence, selectable marker sequences, and optionally, signal sequences and additional restriction sites. An expression vector additionally comprises expression control sequences essential for the transcription and translation of the DNA of the invention. Thus an expression vector refers to a recombinant DNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into a suitable host cell, results in expression of the cloned DNA. Suitable expression vectors are well known in the art and include those that are replicable in eukaryotic and/or prokaryotic cells.

Most expression vectors are capable of replication in at least one class of organisms but

can be transfected into another organism for expression. For example, a vector is cloned in E. coli and then the same vector is transfected into yeast or mammalian cells even though it is not capable of replicating independently of the host cell chromosome. DNA may also be amplified by insertion into the host genome. However, the recovery of genomic DNA encoding hmGluR is more complex than that of exogenously replicated vector because restriction enzyme digestion is required to excise hmGluR DNA. DNA can be amplified by PCR and be directly transfected into the host cells without any replication component.

Advantageously, expression and cloning vector contain a selection gene also referred to as selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics and other toxins, e.g. ampicillin, neomycin, methotrexate or tetracycline, complement auxotrophic deficiencies, or supply critical nutrients not available from complex media.

Since the amplification of the vectors is conveniently done in E. coli, an E. coli genetic marker and an E. coli origin of replication are advantageously included. These can be obtained from E. coli plasmids, such as pBR322, Bluescript vector or a pUC plasmid.

Suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up hmGluR nucleic acid, such as dihydrofolate reductase (DHFR, methotrexate resistance), thymidine kinase, or genes conferring resistance to G418 or hygromycin. The mammalian cell transfectants are placed under selection pressure which only those transfectants are uniquely adapted to survive which have taken up and are expressing the marker.

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to hmGluR2 nucleic acid. Such promoter may be inducible or constitutive. The promoters are operably linked to DNA encoding hmGluR2 by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter sequence into the vector. Both the native hmGluR2 promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of hmGluR DNA. However, heterologous promoters are preferred, because they generally allow for greater transcription and higher yields of expressed hmGluR2 as compared to native hmGluR2 promoter.

Promoters suitable for use with prokaryotic hosts include, for example, the  $\beta$ -lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system and hybrid promoters such as the tac promoter. Their nucleotide sequences have been published, thereby enabling the skilled worker operably to ligate them to DNA encoding hmGluR, using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems will also generally contain a Shine-Delgarno sequence operably linked to the DNA encoding hmGluR2.

HmGluR2 gene transcription from vectors in mammalian host cells may be controlled by promoters compatible with the host cell systems, e.g. promoters derived from the genomes of viruses. Suitable plasmids for expression of the hmGluR subtype of the invention in eukaryotic host cells, particularly mammalian cells, are e.g. cytomegalovirus (CMV) promoter-containing vectors, RSV promoter-containing vectors and SV40 promoter-containing vectors and MMTV LTR promoter-containing vectors. Depending on the nature of their regulation, promoters may be constitutive or regulatable by experimental conditions.

Transcription of a DNA encoding a hmGluR subtype according to the invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector.

The various DNA segments of the vector DNA are operatively linked, i.e. they are contiguous and placed into a functional relationship to each other.

Construction of vectors according to the invention employs conventional ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required. If desired, analysis to confirm correct sequences in the constructed plasmids is performed in a manner known in the art. Suitable methods for constructing expression vectors, preparing in vitro transcripts, introducing DNA into host cells, and performing analyses for assessing hmGluR expression and function are known to those skilled in the art. Gene presence, amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, northern blotting to quantitate the transcription of mRNA, dot blotting (DNA or RNA analysis), in situ hybridization, using an appropriately labelled probe based on a sequence provided herein, binding assays, immunodetection and functional assays. Suitable methods include those described in detail in the Examples. Those skilled in the art

will readily envisage how these methods may be modified, if desired.

The invention further provides host cells capable of producing the hmGluR subtype of the invention and including heterologous (foreign) DNA encoding said subtype.

The nucleic acids of the invention can be expressed in a wide variety of host cells, e.g. those mentioned above, that are transformed or transfected with an appropriate expression vector. The receptor of the invention (or a portion thereof) may also be expressed as a fusion protein. Recombinant cells can then be cultured under conditions whereby the protein (s) encoded by the DNA of the invention is (are) expressed.

Suitable prokaryotes include eubacteria, such as Gram-negative or Gram-positive organisms, such as *E. coli*, e.g. *E. coli* K-12 strains, DH5 $\alpha$  and HB 101, or *Bacilli*. Further host cells suitable for hmGluR encoding vectors include eukaryotic microbes such as filamentous fungi or yeast, e.g. *Saccharomyces cerevisiae*. Higher eukaryotic cells include insect, amphibian and vertebrate cells, particularly mammalian cells, e.g. neuroblastoma cell lines or fibroblast derived cell lines. Examples of preferred mammalian cell lines are e.g. HEK 293 cells, CHO cells, CV1 cells, BHK cells, L cells, LLC-PK-1 cells, GH3 cells, and COS cells. In recent years propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. The host cells referred to in this application comprise cells in in vitro culture as well as cells that are within a host animal.

Suitable host cells for expression of an active recombinant hmGluR2 advantageously express endogenous or recombinant G-proteins. Preferred are cells producing little, if any, endogenous metabotropic glutamate receptor. DNA may be stably incorporated into the cells or may be transiently expressed according to conventional methods.

Stably transfected mammalian cells may be prepared by transfecting cells with an expression vector having a selectable marker gene, and growing the transfected cells under conditions selective for cells expressing the marker gene. To prepare transient transfectants, mammalian cells are transfected with a reporter gene to monitor transfection efficiency.

To produce such stably or transiently transfected cells, the cells should be transfected with a sufficient amount of hmGluR-encoding nucleic acid to form hmGluR of the invention. The precise amounts of DNA encoding hmGluR of the invention may be empirically

determined and optimized for a particular cell and assay.

A DNA of the invention may also be expressed in non-human transgenic animals, particularly transgenic warm-blooded animals. Methods for producing transgenic animals, including mice, rats, rabbits, sheep and pigs, are known in the art and are disclosed, for example by Hammer et al. (Nature 315, 680-683, 1985). An expression unit including a DNA of the invention coding for a hmGluR together with appropriately positioned expression control sequences, is introduced into pronuclei of fertilized eggs. Introduction may be achieved, e.g. by microinjection. Integration of the injected DNA is detected, e.g. by blot analysis of DNA from suitable tissue samples. It is preferred that the introduced DNA be incorporated into the germ line of the animal so that it is passed to the animal's progeny.

Furthermore, a knock-out animal may be developed by introducing a mutation in the mGluR sequence, thereby generating an animal which does not express the functional mGluR2 gene anymore. Such knock-out animal is useful e.g. for studying the role of the metabotropic receptor in metabolism, particularly in normal and disturbed brain function.

More specifically, a knock-out animal may be developed (i.e. an animal that does not express the endogenous mGluR2 gene anymore), in which one introduces a mutated or wild-type hmGluR2 gene. Methods for producing knock-out mice are known in the art. The knock-out animals are useful not only for studying the role of a given metabotropic receptor, as exemplified by published studies (see e.g. F. Conquet et al., Nature 372, 237-243 (1994); ; A. Aiba et al., Cell 79, 365-375 (1994); M. Masu et al., Cell 80, 757-765 (1995)), but also and, in particular, for providing a mammalian animal model with a suitable genetic background for introducing and expressing transgenes encoding the homologous human receptor and/or several of its isoforms. Expression of human counterpart receptors on a homologous gene knock-out background has the unique advantage of excluding differences in efficacies of drugs on a given receptor (in this case mGluR2) caused by species-specific sequence differences in the receptor.

Host cells are transfected or transformed with the above-captioned expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Heterologous DNA may be introduced into host cells by any method known in the art, such as transfection with a vector encoding a heterologous



DNA by the calcium phosphate coprecipitation technique, by electroporation or by lipofectin-mediated. Numerous methods of transfection are known to the skilled worker in the field. Successful transfection is generally recognized when any indication of the operation of this vector occurs in the host cell. Transformation is achieved using standard techniques appropriate to the particular host cells used.

Incorporation of cloned DNA into a suitable expression vector, transfection of eukaryotic cells with a plasmid vector or a combination of plasmid vectors, each encoding one or more distinct genes or with linear DNA, and selection of transfected cells are well known in the art (see, e.g. Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press).

Transfected or transformed cells are cultured using media and culturing methods known in the art, preferably under conditions, whereby hmGluR encoded by the DNA is expressed. The composition of suitable media is known to those in the art, so that they can be readily prepared. Suitable culturing media are also commercially available.

While the DNA provided herein may be expressed in any suitable host cell, e.g. those referred to above, preferred for expression of DNA encoding functional hmGluR are eukaryotic expression systems, particularly mammalian expression systems, including commercially available systems and other systems known to those of skill in the art.

Human mGluR2 DNA of the invention is ligated into a vector, and introduced into suitable host cells to produce transformed cell lines that express hmGluR2 of the invention, or specific combinations of hmGluR subtypes including hmGluR2. The resulting cell line can then be produced in amounts sufficient for reproducible qualitative and quantitative analysis of the effects of a receptor-specific agonist, antagonist or allosteric modulator. Additionally, mRNA may be produced by in vitro transcription of a DNA encoding the subtype of the invention. This mRNA may be injected into *Xenopus* oocytes where the mRNA directs the synthesis of the active receptor subtype.

Alternatively, the subtype-encoding DNA can be directly injected into oocytes. The transfected mammalian cells or injected oocytes may then be employed in a drug screening assay provided hereinafter. Such drugs are useful in diseases associated with pathogenesis of the hmGluR subtype of the invention. Such diseases include diseases resulting from excessive action of glutamate preferentially mediated by hmGluRs, such as stroke, epilepsy and chronic neurodegenerative diseases. Particularly useful for assessing the

specific interaction of compounds with specific hmGluR subtypes are stably transfected cell lines expressing the hmGluR of the invention.

Thus host cells expressing hmGluR of the invention are useful for drug screening and it is a further object of the present invention to provide a method for identifying a compound or signal which modulates the activity of hmGluR2, said method comprising exposing cells containing heterologous DNA encoding hmGluR of the invention, wherein said cells produce functional hmGluR2, to at least one compound or signal whose ability to modulate the activity of said hmGluR is sought to be determined, and thereafter monitoring said cells for changes caused by said modulation. Such an assay enables the identification of agonists, antagonists and allosteric modulators of the hmGluR of the invention.

In a further aspect, the invention relates to an assay for identifying compounds which modulate the activity of hmGluR2, said assay comprising:

- contacting cells expressing an active hmGluR2 and containing heterologous DNA encoding said hmGluR subtype with at least one compound to be tested for its ability to modulate the activity of said receptor, and
- analysing cells for a difference in second messenger level or receptor activity.

In particular, the invention covers an assay for identifying compounds which modulate the activity of hmGluR2, said assay comprising:

- contacting cells expressing active hmGluR2 and containing heterologous DNA encoding said hmGluR subtype with at least one compound to be tested for its ability to modulate the activity of said receptor, and
- monitoring said cells for a resulting change in second messenger activity.

The result obtained in the assay is compared to an assay suitable as a negative control.

Assay methods generally require comparison to various controls. A change in receptor activity or in second messenger level is said to be induced by a test compound if such an effect does not occur in the absence of the test compound. An effect of a test compound on the receptor subtype of the invention is said to be mediated by said receptor if this effect is not observed in cells which do not express the receptor.

As used herein, a compound or signal that modulates the activity of the hmGluR of the invention refers to a compound or signal that alters the response pathway mediated by

hmGluR2 within a cell (as compared to the absence of said hmGluR). A response pathway is activated by an extracellular stimulus, resulting in a change in second messenger concentration or enzyme activity, or resulting in a change of the activity of a membrane-bound protein, such as a receptor or ion channel. A variety of response pathways may be utilized, including for example, the adenylate cyclase response pathway, the phospholipase C/intracellular calcium ion response pathway or a response pathway involving coupling of the receptor to an ion channel. Assays to determine adenylate cyclase activity are well known in the art, and include e.g. the assay disclosed by Nakajima et al., J. Biol. Chem. 267, 2437-2442 (1992))

Thus hmGluR2 expressing cells may be employed for the identification of compounds, particularly low molecular weight molecules capable of acting as glutamate agonists or antagonists. Preferred are low molecular weight molecules of less than 1,000 Dalton. Within the context of the present invention, an agonist is understood to refer to a molecule that is capable of interacting with hmGluR2, thus mimicking the action of L-glutamate. In particular, a glutamate agonist is characterized by its ability to interact with the hmGluR of the invention, and thereby increasing or decreasing the stimulation of a response pathway within a cell. For example, an agonist increases or decreases a measurable parameter within the host cell, such as the concentration of a second messenger, as does the natural ligand increase or decrease said parameter. For example, in a suitable test system, wherein the hmGluR of the invention is negatively coupled to adenylate cyclase, e.g. CHO cells or BHK cells expressing hmGluR2, such an agonist is capable of modulating the function of hmGluR2 such that the intracellular concentration of cAMP is decreased.

By contrast, in situations where it is desirable to tone down the activity of hmGluR2, antagonizing molecules are useful. Within the context of the present invention, an antagonist is understood to refer to a molecule that is capable of interacting with hmGluR2, but which does not stimulate a response pathway within a cell. In particular, glutamate antagonists are generally identified by their ability to interact with hmGluR2 of the invention, and thereby reduce the ability of the natural ligand to stimulate a response pathway within a cell, e.g. by interfering with the binding of L-glutamate to the hmGluR of the invention or by inhibiting other cellular functions required for the activity of the hmGluR. For example, in a suitable assay, e.g. an assay involving CHO cells or BHK cells expressing hmGluR2, a glutamate antagonist is capable of modulating the activity of a hmGluR of the invention such that the ability of the natural ligand to decrease the

intracellular cAMP concentration is weakened. Yet another alternative to achieve an antagonistic effect is to rely on overexpression of antisense hmGluR RNA. Preferred is an agonist or antagonist selectively acting on hmGluR2. Particularly useful is an agonist or antagonist specifically modulating the activity of hmGluR2 without affecting the activity of any other subtype.

An allosteric modulator of a hmGluR of the invention interacts with the receptor protein at another site than L-glutamate, thus acting as agonist or antagonist. Therefore, the screening assays described herein are also useful for detecting an allosteric modulator of a receptor of the invention. For example, an allosteric modulator acting as agonist may enhance the specific interaction between the hmGluR of the invention and L-glutamate. If an allosteric modulator acts as an antagonist, it may e.g. interact with the receptor protein in such a way that binding of the agonist is functionally less effective.

An in vitro assay for a glutamate agonist or antagonist may require that the hmGluR of the invention is produced in sufficient amounts in a functional form using recombinant DNA methods. An assay is then designed to measure a functional property of the hmGluR2 protein, e.g. interaction with a glutamatergic ligand. Production of the hmGluR of the invention is regarded as occurring in sufficient amounts, if activity of said receptor results in a measurable response.

For example, mammalian cells, e.g. HEK293 cells, L cells, CHO-K1 cells, LLC-PK-1 cells or GH3 cells (available e.g. from the American Tissue Type Culture Collection) are adapted to grow in a glutamate reduced, preferably a glutamate free, medium. A hmGluR expression plasmid, e.g. a plasmid described in the Examples, is transiently transfected into the cells, e.g. by calcium-phosphate precipitation (Ausubel, F. M., et al. (1993) Current Protocols in Molecular Biology, Greene and Wiley, USA). Cell lines stably expressing the hmGluR of the invention may be generated e.g. by lipofectin-mediated transfection with hmGluR2 expression plasmids and a plasmid comprising a selectable marker gene, e.g. pSV2-Neo (Southern and Berg, J. Mol. Appl. Genet. 1, 327-341 (1982)), a plasmid vector encoding the G-418 resistance gene. Cells surviving the selection are isolated and grown in the selection medium. Resistant clonal cell lines are analyzed, e.g. for immunoreactivity with subtype-specific antibodies or by assays for hmGluR functional responses following agonist addition. Cells producing the desired hmGluR subtype are used in a method for detecting compounds binding to the hmGluR of the invention or in a method for identifying a glutamate agonist or antagonist.

In a further embodiment, the invention provides a method for identifying compounds binding to hmGluR2, said method comprising employing the hmGluR subtype of the invention in a competitive binding assay. The principle underlying a competitive binding assay is generally known in the art. Briefly, binding assays are performed by allowing the compound to be tested for its hmGluR2 binding capability to compete with a known, suitably labeled, glutamatergic ligand for the binding site at the hmGluR2 target molecule. A suitably labeled ligand is e.g. a radioactively labeled ligand, such as [<sup>3</sup>H]glutamate, or a ligand which can be detected by its optical properties, such as absorbance or fluorescence. After removing unbound ligand and test compound the amount of labeled ligand bound to hmGluR2 is measured. If the amount of labeled ligand is reduced in the presence of the test compound this compound is said to be bound to the target molecule. A competitive binding assay may be performed e.g. with transformed or transfected host cells expressing the hmGluR of the invention or a membraneous cellular fraction comprising the hmGluR of the invention.

Compound bound to the target hmGluR may modulate functional properties of hmGluR2 and may thereby be identified as a glutamate agonist or antagonist in a functional assay.

Functional assays are used to detect a change in the functional activity of hmGluR2 of the invention, i.e. to detect a functional response, e.g. as a result of the interaction of the compound to be tested with said hmGluR. A functional response is e.g. a change (difference) in the concentration of a relevant second messenger, or a change in the activity of another membrane-bound protein influenced by the receptor of the invention within cells expressing functional hmGluR2 (as compared to a negative control). Those of skill in the art can readily identify an assay suitable for detecting a change in the level of an intracellular second messenger indicative of the expression of active hmGluR2 (functional assay). Examples include cAMP assays (see, e.g. Nakajima et al., J. Biol. Chem. 267, 2437-2442 (1992)), cGMP assays (see, e.g. Steiner et al., J. Biol. Chem. 247, 1106-1113 (1972)), phosphatidyl inositol (PI) turnover assays (Nakajima et al., J. Biol. Chem. 267, 2437-2442 (1992)), calcium ion flux assays (Ito et al., J. Neurochem. 56, 531-540 (1991)), arachidonic acid release assays (see, e.g. Felder et al., J. Biol. Chem. 264, 20356-20362 (1989)), and the like.

More specifically, according to the invention a method for detecting a glutamate agonist comprises the steps of (a) exposing a compound to the hmGluR subtype of the invention

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coupled to a response pathway, under conditions and for a time sufficient to allow interaction of the compound with the receptor and an associated response through the pathway, and (b) detecting an increase or decrease in the stimulation of the response pathway resulting from the interaction of the compound with hmGluR2, relative to the absence of the tested compound and therefrom determining the presence of a glutamate agonist.

A method for identifying a glutamate antagonist comprises the steps of (a) exposing a compound in the presence of a known glutamate agonist to hmGluR2 coupled to a response pathway, under conditions and for a time sufficient to allow interaction of the agonist with the receptor and an associated response through the pathway, and (b) detecting an inhibition of the stimulation of the response pathway induced by the agonist, said inhibition resulting from the interaction of the compound with hmGluR2, relative to the stimulation of the response pathway by the glutamate agonist alone and therefrom determining the presence of a glutamate antagonist. Inhibition may be detected, e.g. if the test compound competes with the glutamate agonist for hmGluR2. Compounds which may be screened utilizing such method include blocking antibodies specifically binding to hmGluR2. Furthermore, such an assay is useful for the screening for compounds interacting with L-glutamate. In this case, the agonistic effect is neutralized or reduced, e.g. by binding of the test compound to the agonist, thus affecting agonist interaction with the receptor. Examples are soluble hmGluR fragments comprising part or all of the ligand binding domain.

Preferentially, interaction of an agonist or antagonist with hmGluR2 of the invention denotes binding of the agonist or antagonist to said hmGluR.

As employed herein, conditions and times sufficient for interaction of a glutamate agonist or antagonist candidate with the receptor will vary with the source of the receptor, however, conditions generally suitable for binding occur between about 4°C and about 40°C, preferably between about 4°C and about 37°C, in a buffer solution between 0 and 2 M NaCl, preferably between 0 and 0.9 M NaCl, with 0.1 M NaCl being particularly preferred, and within a pH range of between 5 and 9, preferably between 6.5 and 8. Sufficient time for the binding and response will generally be between about 1 ms and about 24 h after exposure.

Within one embodiment of the present invention, the response pathway is a

membrane-bound adenylate cyclase pathway, and, for an agonist, the step of detecting comprises measuring a reduction or increase, preferably a reduction, in cAMP production by the membrane-bound adenylate cyclase response pathway, relative to the cAMP production in the relevant control setup. For the purpose of the present invention, it is preferred that the reduction or increase in cAMP production be equivalent or greater than the reduction or increase induced by L-glutamate applied at a concentration corresponding to its  $IC_{50}$  value. For an antagonist, the step of detecting comprises measuring in the presence of the antagonist a smaller L-glutamate induced decrease or increase in cAMP production by the membrane-bound adenylate cyclase response pathway, as compared to the cAMP production in the absence of the antagonist. The measurement of cAMP may be performed after cell destruction or by a cAMP sensitive molecular probe loaded into the cell, such as a fluorescent dye, which changes its properties, e.g. its fluorescent properties, upon binding of cAMP.

Cyclic AMP production may be measured using methods well known in the art, including for instance, the method described by Nakajima et al., supra, or using commercially available kits, e.g. kits comprising radiolabeled cAMP, e.g. [ $^{125}I$ ]cAMP or [ $^3H$ ]cAMP. Exemplary kits are the Scintillation Proximity Assay Kit by Amersham, which measures the production of cAMP by competition of iodinated-cAMP with cAMP antibodies, or the Cyclic AMP [ $^3H$ ] Assay Kit by Amersham.

In assay systems using cells expressing hmGluR2 that is negatively coupled to the adenylate cyclase pathway, i.e. which cause a decrease in cAMP upon stimulation and an increase in cAMP upon reduction of stimulation, it is preferred to expose the cells to a compound which reversibly or irreversibly stimulates the adenylate cyclase, e.g. forskolin, or which is a phosphodiesterase inhibitor, such as isobutylmethylxanthine (IBMX), prior to addition of the (potential) receptor agonist or antagonist.

Within another embodiment of the invention, the response pathway is the PI hydrolysis/ $Ca^{2+}$  mobilization pathway. Such an assay for determining the specific interaction of a test compound with the hmGluR subtype of the invention may be functionally linked to changes in the intracellular calcium ion ( $Ca^{2+}$ ) concentration. Several methods for determining a change in the intracellular concentration of  $Ca^{2+}$  are known in the art, e.g. a method involving a calcium ion sensitive fluorescent dye, such as fura-2 (see Grynkiewicz et al., J. Biol. Chem. 260, 3440-3450, 1985), fluo-3 or Indo-1, such as the calcium fluor QuinZ method describe by Charest et al. (J. Biol. Chem. 259, 8679-8773 (1993)), or the

aequorin photoprotein method described by Nakajima-Shimada (Proc. Natl Acad. Sci. USA 88, 6878-6882 (1991)). In one embodiment of the invention, intracellular calcium ion concentration is measured by microfluoremetry in recombinant cells loaded with calcium sensitive fluorescent dyes fluo-3 or fura-2. These measurements may be performed using cells grown in a coverslip allowing the use of an inverted microscope and video-imaging technologies or a fluorescence photometer to measure calcium concentrations at the single cell level. For both approaches, cells transformed with a hmGluR2 expressing plasmid have to be loaded with the calcium indicator. To this end, the growth medium is removed from the cells and replaced with a solution containing fura-2 or fluo-3. The cells are used for calcium measurements preferentially during the following 8h. The microfluorometry follows standard procedures.

Ca<sup>2+</sup> signals resulting from functional interaction of compounds with the target molecule can be transient if the compound is applied for a limited time period, e.g. via a perfusion system. Using transient application several measurements can be made with the same cells allowing for internal controls and high numbers of compounds tested.

Functional coupling of the hmGluR of the invention to Ca<sup>2+</sup> signaling may be achieved, e.g. in CHO cells, by various methods:

- (i) coexpression of a recombinant hmGluR of the invention and a recombinant voltage-gated cation channel, activity of which is functionally linked to the activity of the hmGluR2;
- (ii) expression of a chimeric hmGluR receptor, which directly stimulates the PI/Ca<sup>2+</sup> pathway;
- (iii) coexpression of recombinant hmGluR of the invention with a recombinant Ca<sup>2+</sup>-permeable cAMP dependent cation channel.

In other expression systems functional coupling of hmGluR2 to Ca<sup>2+</sup> signalling may be achieved by transfection of the hmGluR of the invention if these cells naturally express (i) voltage gated Ca channels, activity of which is functionally linked to activity of mGluRs or (ii) Ca<sup>2+</sup>-permeable cAMP dependent ion channels. For example, GH3 cells which naturally express voltage-gated Ca channels, directly allow application of Ca<sup>2+</sup> assays to test for hmGluR2 functional activity by cotransfection of hmGluRs.

Further cell-based screening assays can be designed e.g. by constructing cell lines in which the expression of a reporter protein, i.e. an easily assayable protein, such as



$\beta$ -galactosidase, chloramphenicol acetyltransferase (CAT) or luciferase, is dependent on the function of a hmGluR of the invention. For example, a DNA construct comprising a cAMP response element is operably linked to a DNA encoding luciferase. The resulting DNA construct comprising the enzyme DNA is stably transfected into a host cell. The host cell is then transfected with a second DNA construct containing a first DNA segment encoding the hmGluR of the invention operably linked to additional DNA segments necessary for the expression of the receptor. For example, if binding of an agonist to the hmGluR of the invention results in decreased cAMP levels, the expression of luciferase is induced or decreases, depending on the promoter chosen. The luciferase is exposed to luciferin, and the photons emitted during oxidation of luciferin by the luciferase is measured.

The drug screening assays provided herein will enable identification and design of receptor subtype-specific compounds, particularly ligands binding to hmGluR2, eventually leading to the development of a disease-specific drug. If designed for a very specific interaction with only one particular hmGluR subtype (or a predetermined selection of hmGluR subtypes) such a drug is most likely to exhibit fewer unwanted side effects than a drug identified by screening with cells that express a(n) (unknown) variety of receptor subtypes. Also, testing of the single receptor subtype of the invention or specific combinations of different receptor subtypes with a variety of potential agonists or antagonists provides additional information with respect to the function and activity of individual hmGluR2 protein and should lead to the identification and design of compounds that are capable of very specific interaction with one or more receptor subtypes.

In another embodiment the invention provides polyclonal and monoclonal antibodies generated against hmGluR2. Such antibodies may be useful e.g. for immunoassays including immunohistochemistry as well as diagnostic and therapeutic applications. For example, antibodies specific for the extracellular domain, or portions thereof, of hmGluR2 can be applied for blocking the endogenous hmGluR subtype.

The antibodies of the invention can be prepared according to methods well known in the art using as antigen the hmGluR of the invention, a fragment thereof or a cell expressing said subtype or fragment. The antigen may represent the active or inactive form of the receptor of the invention. Antibodies may be capable of distinguishing between the active or inactive form. Factors to consider in selecting subtype fragments as antigens (either as

synthetic peptide or as fusion protein) include antigenicity, accessibility (i.e. extracellular and cytoplasmic domains) and uniqueness to the particular subtype.

Particularly useful are antibodies selectively recognizing and binding to hmGluR2. The antibodies of the invention can be administered to a subject in need thereof employing standard methods. One of skill in the art can readily determine dose forms, treatment regimens etc, depending on the mode of administration employed.

The invention particularly relates to the specific embodiments as described in the Examples which serve to illustrate the present invention but should not be construed as a limitation thereof.

Abbreviations: hmGluR = human metabotropic glutamate receptor, nt=nucleotide

#### Example 1: Cloning and expression of cDNA encoding hmGluR2

##### 1.1 cDNA cloning from human brain

N-terminal and C-terminal fragments of the rat mGluR2 cDNA (fragment nt 192 to 518 and fragment nt 1983- 2810, Tanabe et al., Neuron 8, 169-179 (1992)) are generated by PCR from single stranded rat forebrain cDNA.

Single strand cDNA synthesis is carried out with 1 µg rat forebrain poly(A)<sup>+</sup> RNA, 80 U M-MLV reverse transcriptase (BRL), 25 mM Tris-HCl pH 8.3, 37.5 mM KCl, 1.5 mM MgCl<sub>2</sub>, 10 mM dithiothreitol, 1 mM each dATP, dCTP, dGTP, dTTP, 50 mg/ml oligo-dT<sub>12-18</sub> (Pharmacia), and 2 U RNasin (Promega) at 37°C for 60 min. The 5' and 3' primers used for PCR are ATGGAATCACTGCTTGGGT/TGAGGCAGG-CACAAAGTCCA for the N-terminal fragment (nucleotides 192 to 518) and GTCAAG-GCTTCCGGTCGGGA/TCAAAGCGACGACGTTGTTGA for the C-terminal fragment (nucleotides 1983 to 2810), respectively. PCR reactions are performed using the GeneAMP DNA amplification kit (Perkin Elmer Cetus) under the following conditions: 93°C for 0.5 min, 56° C for 1.5 min, and 72°C for 3 min for 40 cycles. The amplified DNAs are gel purified, cloned into the SmaI site of pBluescript SK, and characterized by DNA sequencing (Sequenase T7 polymerase Kit, United States Biochemicals). 2x 10<sup>6</sup> plaques of human fetal brain and human adult hippocampus cDNA libraries, constructed in Lambda-ZAPII (Stratagene) from oligo-(dT) and randomly primed poly(A)<sup>+</sup> RNA, are screened sequentially with N- and C-terminal rat mGluR2 probes. Metabotropic GluR2 probes are generated by random priming of gel purified fragments

using [ $\alpha$ - $^{32}$ P]dCTP. Hybridizations are carried out overnight at 60° C in 5x SSC/5x Denhardt's/50 mM Na<sub>2</sub>HPO<sub>4</sub>/10 mM EDTA/1% SDS/50 µg/ml Herring Testis DNA/20 µg/ml yeast RNA. Washes are done for 30 min each at 25 C in 5x SSC/0.2% SDS, 2x SSC/0.2% SDS, and 1x SSC/0.2% SDS. Five plaques hybridizing to the N- and C-terminal rat mGluR2 fragments are purified by a second and third round of screening and five cDNA inserts are rescued into Bluescript SK phagemids by in vivo excision. cDNA inserts are characterized by restriction enzyme mapping and DNA sequencing. The cDNA clones show restriction maps, which differed only at the very 5' and 3' end. The largest cDNA clone, hmGluR2.1, contains a 4.1 kb KpnI/NotI fragment. The entire coding sequence is sequenced on both strands. The DNA sequence coding for the hmGluR2 protein and the deduced amino acid sequence are set forth in SEQ ID NOs. 1 and 2, respectively.

**1.2 Construction of hmGluR2 expression construct and expression in mammalian cells**  
The 4.1 kb insert of the cDNA hmGluR2.1 is cloned downstream of the mouse CMV promoter in the mammalian expression vector pSMC (Asselbergs and Grand, 1993) into the blunt-ended NotI/KpnI sites resulting in the expression construct pSMChmGluR2s. Chinese hamster ovary cells (CHO-K1) are cotransfected with pSMChmGluR2s and pSV2-Neo (Southern and Berg, Journal of Molecular and Applied Genetics 1, 327-341 (1982)) using lipofectin mediated gene transfer (Gibco-BRL). Thirty-two G-418 resistant clonal cell lines are isolated and analyzed for mGluR2 protein expression by immunoreactivity with an anti-hmGluR2 antibody (immunodetection, see infra) and functional responses following agonist addition via cAMP radioimmuno assay (see infra).

**Example 2: Immunodetection of hmGluR2 protein expression with subtype-specific hmGluR antibodies**

HmGluR2 expression is analyzed by immunocytochemistry with subtype-specific hmGluR antibodies (see Example 5). 1 to 3 days after transfection cells are washed twice with phosphate buffered saline (PBS), fixed with PBS/4% paraformaldehyde for 10 min and washed with PBS. Cells are permeabilized with PBS/0.4 % Triton X-100, followed by washing with PBS/10 mM glycine, and PBS. Cells are blocked with PBSTB (1x PBS/0.1% Triton X-100/1 % BSA) for 1 h and subsequently incubated with immunopurified hmGluR antiserum (0.5 - 2.0 µg/ml in PBSTB) for 1 h. After three washes with PBS, cells are incubated for 1 h with alkaline peroxidase conjugated goat anti-rabbit IgG (1:200 in PBSTB; Jackson Immuno Research). Cells are washed three

times with PBS and immunoreactivity is detected with 0.4 mg/ml naphtholphosphate (Biorad)/1 mg/ml Fast Red (Biorad)/10 mM Levamisole (Sigma)/100 mM Tris/HCl pH 8.8/100 mM NaCl/50 mM MgCl<sub>2</sub>. The staining reaction is stopped after 15 min by subsequent washing with PBS. Four cell lines, each homogenously expressing hmGluR2 are identified by immunostaining.

**Example 3: Use of stable cell lines expressing hmGluR2 for the screening of modulators of receptor activity**

Stable cell lines expressing hmGluR2 are used to screen for agonists, antagonists and allosteric modulators. Such compounds are identified by binding studies employing [<sup>3</sup>H]glutamate and/or measurement of changes in intracellular second messenger levels ([cAMP], [Ca<sup>2+</sup>]).

**3.1 cAMP radioimmunoassay**

Ligand binding and agonist-induced depression of forskolin stimulated cAMP accumulation (changes in the intracellular cAMP concentration) are analyzed by cAMP radioimmunoassay (Amersham). Cells are seeded in 12-well plates at a density of 0.5-2.0 x 10<sup>5</sup> cells per well and grown for 2 to 4 days until a confluent layer of cells is obtained. Cells are washed twice with PBS and incubated for 20 min in PBS containing 1 mM 3-isobutyl-1-methylxanthine (IBMX). Cells are incubated with fresh PBS containing 10 μM forskolin, 1 mM IBMX and a known hmGluR agonist for 20 min. The agonistic effect is stopped and cAMP produced by the cells is released by adding 1 ml of ethanol-water-HCl mix (100 ml of ethanol, 50 ml of water, 1 ml of 1 M HCl) after having aspirated the drug containing medium. cAMP levels are determined by a cAMP radioimmunoassay involving [<sup>3</sup>H] cAMP (Amersham).

HmGluR2 is negatively coupled to adenylate cyclase when expressed in CHO cells. Agonist binding leads to an inhibition of forskolin induced cAMP accumulation. The rank order of agonist potencies is (2S,3S,4S)-α-(carboxycyclopropyl)-glycine > (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid = L-glutamate > quisqualate.

**3.2 Measurement of intracellular [Ca<sup>2+</sup>]**

Cells transformed with a hmGluR2 expression plasmid, e.g. the above expression plasmid, are loaded with a calcium sensitive fluorescent dye such as fura-2 or fluoro-3. To achieve this cells are plated in single wells, single wells containing a coverslip, or 96-well plates and grown for 1 to 5 days until a 50-100 % confluent layer of cells is obtained. Wells are

washed three times with a balance salt solution (BBS) and incubated for 1h in BBS followed by three additional washings with BBS. Then cells are incubated for 20 to 60 min in a solution containing 50 µg fura-2-AM (or fluoro3-AM) (Molecular Probes, Inc.) 4.99 ml BBS, 75 µl DMSO and 6.25 µg Pluronic (Molecular Probes, Inc.). The cells are washed 3 times with BBS containing 2 mg/ml bovine albumin followed by three washes in BBS. After allowing recovery of the cells for at least 10 min they are used for microfluorometric measurements of  $[Ca^{2+}]$ .

Cells are transferred to an apparatus for fluometry such as an inverted microscope, a spectrofluometer or a fluorescence reader. Fluorescence of the calcium indicator (e.g. fura-2 or fluoro-3) is induced by illumination with light of a wavelength covered by the excitation spectrum of the dye (fura-2: 340/380 nm, fluoro-3 480 nm). An increase in intracellular free calcium ion concentration is monitored as an increase of fura-2 or fluoro-3 fluorescence excited at 340 nm and 480 nm, respectively, or a decrease of fura-2 fluorescence excited at 380 nm.

As a positive control, L-glutamate is applied at a concentration corresponding to its  $EC_{50}$  value onto the cells, thereby inducing a measurable increase in the intracellular calcium ion concentration. A test compound is said to be an agonist if it induces a  $Ca^{2+}$  signal comparable to that induced by glutamate. A test compound is said to be an antagonist if the glutamate induced calcium signal is smaller in the presence of the test compound than in the absence of the test compound.

#### Example 4: Chimeric hmGluR2

Intracellular domains of mGluR1, particularly the second intracellular loop (i2) and the C-terminal region, have been shown to be critical for binding of G-proteins, which activate the phospholipase C/ $Ca^{2+}$  signaling pathway, without changing the pharmacological profile of the receptor (Pin et al., EMBO J. 13, 342-348, (1994)).

Conventional PCR mutagenesis techniques are used to exchange intracellular domains of hmGluRs 2 with corresponding domains of hmGluR1. Stable CHO cell lines are generated with hmGluR2/1 chimeric expression constructs allowing to analyze the influence of modulators of receptor activity (hmGluR2) using  $Ca^{2+}$ -dependent assays.

(i) The cDNA clone hmGluR2.1 is used for the construction of chimera.

(ii) The transmembrane region of hmGluR1 is cloned by PCR using primers derived from Masu et al., 1991, supra. The oligonucleotide with the sequence

5'-TATCTTGAGTGGAGTGACATAG-3'

(corresponding to nt 1753 to 1774 of the Masu sequence) is used as sense primer. The

antisense primer has the sequence

5'-ACTGCGGACGTTCTCTCAGG-3'

corresponding to nt 2524 to 2544 of the Masu sequence. The C-terminal end of splice variants 1a, 1b and 1c is cleaved by PCR using primers derived from Masu et al., 1991, Tanabe et al., 1992, supra, and Pin et al. (Proc. Natl. Acad. Sci, USA, 89, 10331-10335 (1992)), respectively. The oligonucleotide having the sequence

5'-AAACCTGAGAGGAACGTCCGCAG-3'

(corresponding to nt 2521 to nt 2543 of the Masu sequence) is used as sense primer. The oligonucleotides having the sequences

5'-CTACAGGGTGGGAAGAGCTTTGCTT-3' corresponding to nt 3577 to 3600 of the Masu sequence,

5'-TCAAAGCTGCGCATGTGCCGACGG-3' corresponding to nt 2698 to 2721 of the Tanabe sequence, and

5'-TCAATAGACAGTGTTTTGGCGGTC-3' corresponding to nt 2671 to 2694 of the Pin sequence are used as antisense primers for hmGluR1a, 1b and 1c, respectively.

The PCR fragment is cloned into pBluescript II and sequenced completely.

(iii) A chimeric cDNA fragment wherein the i2-loop of hmGluR2 (nt 1966-2037 of SEQ ID NO:1) is replaced with the corresponding sequences of hmGluR1 is generated by PCR (as described in Pin et al., 1994, supra). The fragment is digested with Bsu36I and DraIII which cut at unique restriction sites flanking the i2-loop. The chimeric Bsu36I/DraIII fragment is exchanged for the Bsu36I/DraIII fragment of clone hmGluR2.1.

(iv) Additional replacement of the C-terminal domain of hmGluR2 with the corresponding sequences of the above mentioned hmGluR1 splice variants is achieved by using the unique restriction sites DraIII and KpnI flanking the C-terminal end of hmGluR2.

(v) The resulting chimeric hmGluR2/hmGluR1 cDNA is sequenced and digested with KpnI and NotI, thereby releasing the complete cDNA from pBluescript. For stable expression in CHO cells, the chimeric cDNA is blunt-ended and cloned into blunt-ended NotI site of the mammalian expression vector pCMV-T7-2 for stable expression of chimeric hmGluR2/1 receptor in CHO cells.

#### Example 5: Generation and application of anti-hmGluR2 antibodies

Peptides corresponding to the deduced C-terminal amino acid sequences of hmGluR2 are synthesized and coupled to ovalbumin or Tentagel. Polyclonal antisera are raised in rabbits. Human mGluR2 specific antibodies are purified from the antisera by immunoaffinity chromatography on peptide columns. The hmGluR2 specific antibodies are characterized by ELISA and immunoblotting with glutathione-S-transferase/hmGluR

fusion proteins (produced in *E. coli*) or human brain extracts. Antibodies specific for hmGluR2 are used to detect hmGluR2 receptors in transfected cells and to analyze the cellular and subcellular expression pattern of the hmGluR2 proteins in tissue sections of human brain material.

Antibodies are raised against different hmGluR2-specific peptides consisting of 20 amino acids and fusion proteins expressed in *E. coli*. Peptides are synthesized by solid-phase synthesis, coupled to keyhole limpet hemocyanin (KLH) or ovalbumin with glutaraldehyde. PCR fragments containing the entire putative intracellular C-terminal fragment of hmGluR2 are cloned as BamHI/EcoRI fragments into the *E. coli* expression plasmid pGEX-2T (Guan and Dixon, Analytical Biochemistry 192, 262-267 (1991)) generating glutathione-S-transferase(GST)/hmGluR fusion genes. *E. coli* DH5a cells (Gibco-BRL) carrying expression plasmids with GST/hmGluR fusion genes are grown overnight at 37°C in LB medium/100 mg/ml ampicillin. The cultures are diluted 1:30 in LB and grown for 2 h at 30°C. Expression of fusion proteins is induced by treatment with 0.1 mM isopropyl-b-D-thiogalactopyranoside for 3 h at 30°C. Cells are harvested by centrifugation at 5,000 x g. The fusion protein is isolated using glutathione affinity chromatography.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: CIBA-GEIGY AG
- (B) STREET: Klybeckstr. 141
- (C) CITY: Basel
- (E) COUNTRY: SCHWEIZ
- (F) POSTAL CODE (ZIP): 4002
- (G) TELEPHONE: +41 61 69 11 11
- (H) TELEFAX: + 41 61 696 79 76
- (I) TELEX: 962 991

(ii) TITLE OF INVENTION: Glutamate Receptor

(iii) NUMBER OF SEQUENCES: 2

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2618 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2618

(D) OTHER INFORMATION: /product= "hmGluR2"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | GGA | TCG | CTG | CTT | GCG | CTC | CTG | GCA | CTG | CTG | CCG | CTG | TGG | GGT | GCT |
| Met | Gly | Ser | Leu | Leu | Ala | Leu | Leu | Ala | Leu | Leu | Pro | Leu | Trp | Gly | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GTG | GCT | GAG | GGC | CCA | GCC | AAG | AAG | GTG | CTG | ACC | CTG | GAG | GGA | GAC | TTG |
| Val | Ala | Glu | Gly | Pro | Ala | Lys | Lys | Val | Leu | Thr | Leu | Glu | Gly | Asp | Leu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GTG | CTG | GGT | GGG | CTG | TTC | CCA | GTG | CAC | CAG | AAG | GGC | GGC | CCA | GCA | GAG |
| Val | Leu | Gly | Gly | Leu | Phe | Pro | Val | His | Gln | Lys | Gly | Gly | Pro | Ala | Glu |
|     |     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GAC | TGT | GGT | CCT | GTC | AAT | GAG | CAC | CGT | GGC | ATC | CAG | CGC | CTG | GAG | GCC |
| Asp | Cys | Gly | Pro | Val | Asn | Glu | His | Arg | Gly | Ile | Gln | Arg | Leu | Glu | Ala |
|     | 50  |     |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | CTT | TTT | GCA | CTG | GAC | CGC | ATC | AAC | CGT | GAC | CCG | CAC | CTG | CTG | CCT |
| Met | Leu | Phe | Ala | Leu | Asp | Arg | Ile | Asn | Arg | Asp | Pro | His | Leu | Leu | Pro |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GGC | GTG | CGC | CTG | GGT | GCA | CAC | ATC | CTC | GAC | AGT | TGC | TCC | AAG | GAC | ACA |
| Gly | Val | Arg | Leu | Gly | Ala | His | Ile | Leu | Asp | Ser | Cys | Ser | Lys | Asp | Thr |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

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CAT GCG CTG GAG CAG GCA CTG GAC TTT GTG CGT GCC TCA CTC AGC CGT  
 His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg  
                             100                            105                            110

GGT GCT GAT GGA TCA CGC CAC ATC TGC CCC GAC GGC TCT TAT GCG ACC  
 Gly Ala Asp Gly Ser Arg His Ile Cys Pro Asp Gly Ser Tyr Ala Thr  
                             115                            120                            125

CAT GGT GAT GCT CCC ACT GCC ATC ACT GGT GTT ATT GGC GGT TCC TAC  
 His Gly Asp Ala Pro Thr Ala Ile Thr Gly Val Ile Gly Gly Ser Tyr  
                             130                            135                            140

AGT GAT GTC TCC ATC CAG GTG GCC AAC CTC TTG AGG CTA TTT CAG ATC  
 Ser Asp Val Ser Ile Gln Val Ala Asn Leu Leu Arg Leu Phe Gln Ile  
                             145                            150                            155                            160

CCA CAG ATT AGC TAC GCC TCT ACC AGT GCC AAG CTG AGT GAC AAG TCC  
 Pro Gln Ile Ser Tyr Ala Ser Thr Ser Ala Lys Leu Ser Asp Lys Ser  
                             165                            170                            175

CGC TAT GAC TAC TTT GCC CGC ACA GTG CCT CCT GAC TTC TTC CAA GCC  
 Arg Tyr Asp Tyr Phe Ala Arg Thr Val Pro Pro Asp Phe Phe Gln Ala  
                             180                            185                            190

AAG GCC ATG GCT GAG ATT CTC CGC TTC TTC AAC TGG ACC TAT GTG TCC  
 Lys Ala Met Ala Glu Ile Leu Arg Phe Phe Asn Trp Thr Tyr Val Ser  
                             195                            200                            205

ACT GAG GCC TCT GAG GGC GAC TAT GGC GAG ACA GGC ATT GAG GCC TTT  
 Thr Glu Ala Ser Glu Gly Asp Tyr Gly Glu Thr Gly Ile Glu Ala Phe  
                             210                            215                            220

GAG CTA GAG GCT CGT GCC CGC AAC ATC TGT GTG GCC ACC TCG GAG AAA  
 Glu Leu Glu Ala Arg Ala Arg Asn Ile Cys Val Ala Thr Ser Glu Lys  
                             225                            230                            235                            240

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|   |      |
|---|------|
| GTG GGC CGT GCC ATG AGC CGC GCG GCC TTT GAG GGT GTG GTG CGA GCC | 768  |
| Val Gly Arg Ala Met Ser Arg Ala Ala Phe Glu Gly Val Val Arg Ala |      |
| 245 250 255   |      |
| CTG CTG CAG AAG CCC AGT GCC CGC GTG GCT GTC CTG TTC ACC CGT TCT | 816  |
| Leu Leu Gln Lys Pro Ser Ala Arg Val Ala Val Leu Phe Thr Arg Ser |      |
| 260 265 270   |      |
| GAG GAT GCC CGG GAG CTG CTT GCT GCC AGC CAG CGC CTC AAT GCC AGC | 864  |
| Glu Asp Ala Arg Glu Leu Leu Ala Ala Ser Gln Arg Leu Asn Ala Ser |      |
| 275 280 285   |      |
| TTC ACC TGG GTG GCC AGT GAT GGT TGG GGG GCC CTG GAG AGT GTG GTG | 912  |
| Phe Thr Trp Val Ala Ser Asp Gly Trp Gly Ala Leu Glu Ser Val Val |      |
| 290 295 300   |      |
| GCA GGC AGT GAG GGG GCT GCT GAG GGT GCT ATC ACC ATC GAG CTG GCC | 960  |
| Ala Gly Ser Glu Gly Ala Ala Glu Gly Ala Ile Thr Ile Glu Leu Ala |      |
| 305 310 315 320   |      |
| TCC TAC CCC ATC AGT GAC TTT GCC TCC TAC TTC CAG AGC CTG GAC CCT | 1008 |
| Ser Tyr Pro Ile Ser Asp Phe Ala Ser Tyr Phe Gln Ser Leu Asp Pro |      |
| 325 330 335   |      |
| TGG AAC AAC AGC CGG AAC CCC TGG TTC CGT GAA TTC TGG GAG CAG AGG | 1056 |
| Trp Asn Asn Ser Arg Asn Pro Trp Phe Arg Glu Phe Trp Glu Gln Arg |      |
| 340 345 350   |      |
| TTC CGC TGC AGC TTC CGG CAG CGA GAC TGC GCA GCC CAC TCT CTC CGG | 1104 |
| Phe Arg Cys Ser Phe Arg Gln Arg Asp Cys Ala Ala His Ser Leu Arg |      |
| 355 360 365   |      |
| GCT GTG CCC TTT GAA CAG GAG TCC AAG ATC ATG TTT GTG GTC AAT GCA | 1152 |
| Ala Val Pro Phe Glu Gln Glu Ser Lys Ile Met Phe Val Val Asn Ala |      |
| 370 375 380   |      |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|
| GTG | TAC | GCC | ATG | GCC | CAT | GCG | CTC | CAC | AAC | ATG | CAC | CGT | GCC | CTC | TGC | 1 |
| Val | Tyr | Ala | Met | Ala | His | Ala | Leu | His | Asn | Met | His | Arg | Ala | Leu | Cys |   |
| 385 |     |     |     |     |     | 390 |     |     |     | 395 |     |     |     |     | 400 |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CCC | AAC | ACC | ACC | CGG | CTC | TGT | GAC | GCG | ATG | CGG | CCA | GTT | AAC | GGG | CGC | 1 |
| Pro | Asn | Thr | Thr | Arg | Leu | Cys | Asp | Ala | Met | Arg | Pro | Val | Asn | Gly | Arg |   |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     | 415 |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CGC | CTC | TAC | AAG | GAC | TTT | GTG | CTC | AAC | GTC | AAG | TTT | GAT | GCC | CCC | TTT | 1 |
| Arg | Leu | Tyr | Lys | Asp | Phe | Val | Leu | Asn | Val | Lys | Phe | Asp | Ala | Pro | Phe |   |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CGC | CCA | GCT | GAC | ACC | CAC | AAT | GAG | GTC | CGC | TTT | GAC | CGC | TTT | GGT | GAT | 1 |
| Arg | Pro | Ala | Asp | Thr | His | Asn | Glu | Val | Arg | Phe | Asp | Arg | Phe | Gly | Asp |   |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| GGT | ATT | GGC | CGC | TAC | AAC | ATC | TTC | ACC | TAT | CTG | CGT | GCA | GGC | AGT | GGG | 1 |
| Gly | Ile | Gly | Arg | Tyr | Asn | Ile | Phe | Thr | Tyr | Leu | Arg | Ala | Gly | Ser | Gly |   |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CGC | TAT | CGC | TAC | CAG | AAG | GTG | GGC | TAC | TGG | GCA | GAA | GGC | TTG | ACT | CTG | 1 |
| Arg | Tyr | Arg | Tyr | Gln | Lys | Val | Gly | Tyr | Trp | Ala | Glu | Gly | Leu | Thr | Leu |   |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| GAC | ACC | AGC | CTC | ATC | CCA | TGG | GCC | TCA | CCG | TCA | GCC | GGC | CCC | CTG | GCC | 1 |
| Asp | Thr | Ser | Leu | Ile | Pro | Trp | Ala | Ser | Pro | Ser | Ala | Gly | Pro | Leu | Ala |   |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| GCC | TCT | CGC | TGC | AGT | GAG | CCC | TGC | CTC | CAG | AAT | GAG | GTG | AAG | AGT | GTG | 1 |
| Ala | Ser | Arg | Cys | Ser | Glu | Pro | Cys | Leu | Gln | Asn | Glu | Val | Lys | Ser | Val |   |
|     |     |     | 500 |     |     |     |     | 505 |     |     |     |     | 510 |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CAG | CCG | GGC | GAA | GTC | TGC | TGC | TGG | CTC | TGC | ATT | CCG | TGC | CAG | CCC | TAT | : |
| Gln | Pro | Gly | Glu | Val | Cys | Cys | Trp | Leu | Cys | Ile | Pro | Cys | Gln | Pro | Tyr |   |
|     |     |     | 515 |     |     |     |     | 520 |     |     |     |     | 525 |     |     |   |

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|   |      |
|---|------|
| GAG TAC CGA TTG GAC GAA TTC ACT TGC GCT GAT TGT GGC CTG GGC TAC | 1632 |
| Glu Tyr Arg Leu Asp Glu Phe Thr Cys Ala Asp Cys Gly Leu Gly Tyr |      |
| 530 535 540   |      |
| TGG CCC AAT GCC AGC CTG ACT GGC TGC TTC GAA CTG CCC CAG GAG TAC | 1680 |
| Trp Pro Asn Ala Ser Leu Thr Gly Cys Phe Glu Leu Pro Gln Glu Tyr |      |
| 545 550 555 560   |      |
| ATC CGC TGG GGC GAT GCC TGG GCT GTG GGA CCT GTC ACC ATC GCC TGC | 1728 |
| Ile Arg Trp Gly Asp Ala Trp Ala Val Gly Pro Val Thr Ile Ala Cys |      |
| 565 570 575   |      |
| CTC GGT GCC CTG GCC ACC CTG TTT GTG CTG GGT GTC TTT GTG CGG CAC | 1776 |
| Leu Gly Ala Leu Ala Thr Leu Phe Val Leu Gly Val Phe Val Arg His |      |
| 580 585 590   |      |
| AAT GCC ACA CCA GTG GTC AAG GCC TCA GGT CGG GAG CTC TGC TAC ATC | 1824 |
| Asn Ala Thr Pro Val Val Lys Ala Ser Gly Arg Glu Leu Cys Tyr Ile |      |
| 595 600 605   |      |
| CTG CTG GGT GGT GTC TTC CTC TGC TAC TGC ATG ACC TTC ATC TTC ATT | 1872 |
| Leu Leu Gly Gly Val Phe Leu Cys Tyr Cys Met Thr Phe Ile Phe Ile |      |
| 610 615 620   |      |
| GCC AAG CCA TCC ACG GCA GTG TGT ACC TTA CGG CGT CTT GGT TTG GGC | 1920 |
| Ala Lys Pro Ser Thr Ala Val Cys Thr Leu Arg Arg Leu Gly Leu Gly |      |
| 625 630 635 640   |      |
| ACT GCC TTC TCT GTC TGC TAC TCA GCC CTG CTC ACC AAG ACC AAC CGC | 1968 |
| Thr Ala Phe Ser Val Cys Tyr Ser Ala Leu Leu Thr Lys Thr Asn Arg |      |
| 645 650 655   |      |
| ATT GCA CGC ATC TTC GGT GGG GCC CGG GAG GGT GCC CAG CGG CCA CGC | 2016 |
| Ile Ala Arg Ile Phe Gly Gly Ala Arg Glu Gly Ala Gln Arg Pro Arg |      |
| 660 665 670   |      |

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---|
| TTC | ATC | AGT | CCT | GCC | TCA | CAG | GTG | GCC | ATC | TGC | CTG | GCA | CTT | ATC | TCG | 2 |
| Phe | Ile | Ser | Pro | Ala | Ser | Gln | Val | Ala | Ile | Cys | Leu | Ala | Leu | Ile | Ser |   |
|     |     | 675 |     |     |     |     | 680 |     |     |     |     | 685 |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| GGC | CAG | CTG | CTC | ATC | GTG | GTC | GCC | TGG | CTG | GTG | GTG | GAG | GCA | CCG | GGC | 2 |
| Gly | Gln | Leu | Leu | Ile | Val | Val | Ala | Trp | Leu | Val | Val | Glu | Ala | Pro | Gly |   |
|     |     | 690 |     |     |     |     | 695 |     |     |     |     | 700 |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| ACA | GGC | AAG | GAG | ACA | GCC | CCC | GAA | CGG | CGG | GAG | GTG | GTG | ACA | CTG | CGC | 2 |
| Thr | Gly | Lys | Glu | Thr | Ala | Pro | Glu | Arg | Arg | Glu | Val | Val | Thr | Leu | Arg |   |
| 705 |     |     |     |     |     | 710 |     |     |     | 715 |     |     |     |     | 720 |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| TGC | AAC | CAC | CGC | GAT | GCA | AGT | ATG | TTG | GGC | TCG | CTG | GCC | TAC | AAT | GTG | 2 |
| Cys | Asn | His | Arg | Asp | Ala | Ser | Met | Leu | Gly | Ser | Leu | Ala | Tyr | Asn | Val |   |
|     |     |     |     | 725 |     |     |     |     | 730 |     |     |     |     | 735 |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CTC | CTC | ATC | GCG | CTC | TGC | ACG | CTT | TAT | GCC | TTC | AAT | ACT | CGC | AAG | TGC | 2 |
| Leu | Leu | Ile | Ala | Leu | Cys | Thr | Leu | Tyr | Ala | Phe | Asn | Thr | Arg | Lys | Cys |   |
|     |     |     | 740 |     |     |     |     |     | 745 |     |     |     |     | 750 |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| CCC | GAA | AAC | TTC | AAC | GAG | GCC | AAG | TTC | ATT | GGC | TTC | ACC | ATG | TAC | ACC | 2 |
| Pro | Glu | Asn | Phe | Asn | Glu | Ala | Lys | Phe | Ile | Gly | Phe | Thr | Met | Tyr | Thr |   |
|     |     | 755 |     |     |     |     | 760 |     |     |     |     | 765 |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| ACC | TGC | ATC | ATC | TGG | CTG | GCA | TTG | TTG | CCC | ATC | TTC | TAT | GTC | ACC | TCC | 2 |
| Thr | Cys | Ile | Ile | Trp | Leu | Ala | Leu | Leu | Pro | Ile | Phe | Tyr | Val | Thr | Ser |   |
|     |     | 770 |     |     |     | 775 |     |     |     |     |     | 780 |     |     |     |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| AGT | GAC | TAC | CGG | GTA | CAG | ACC | ACC | ACC | ATG | TGC | GTG | TCA | GTC | AGC | CTC | 2 |
| Ser | Asp | Tyr | Arg | Val | Gln | Thr | Thr | Thr | Met | Cys | Val | Ser | Val | Ser | Leu |   |
| 785 |     |     |     |     |     | 790 |     |     |     | 795 |     |     |     |     | 800 |   |
|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |   |
| AGC | GGC | TCC | GTG | GTG | CTT | GGC | TGC | CTC | TTT | GCG | CCC | AAG | CTG | CAC | ATC | 2 |
| Ser | Gly | Ser | Val | Val | Leu | Gly | Cys | Leu | Phe | Ala | Pro | Lys | Leu | His | Ile |   |
|     |     |     |     |     | 805 |     |     |     | 810 |     |     |     |     | 815 |     |   |

ATC CTC TTC CAG CCG CAG AAG AAC GTG GTT AGC CAC CGG GCA CCC ACC 2496  
 Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr  
                   820                                  825                                  830

AGC CGC TTT GGC AGT GCT GCT GCC AGG GCC AGC TCC AGC CTT GGC CAA 2544  
 Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln  
                   835                                  840                                  845

GGG TCT GGC TCC CAG TTT GTC CCC ACT GTT TGC AAT GGC CGT GAG GTG 2592  
 Gly Ser Gly Ser Gln Phe Val Pro Thr Val Cys Asn Gly Arg Glu Val  
                   850                                  855                                  860

GTG GAC TCG ACA ACG TCA TCG CTT TG 2619  
 Val Asp Ser Thr Thr Ser Ser Leu  
                   865                                  870

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 872 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Gly Ser Leu Leu Ala Leu Leu Ala Leu Leu Pro Leu Trp Gly Ala  
   1                                  5                                  10                                  15

Val Ala Glu Gly Pro Ala Lys Lys Val Leu Thr Leu Glu Gly Asp Leu  
                   20                                  25                                  30

Val Leu Gly Gly Leu Phe Pro Val His Gln Lys Gly Gly Pro Ala Glu  
                   35                                  40                                  45

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Asp Cys Gly Pro Val Asn Glu His Arg Gly Ile Gln Arg Leu Glu Ala  
50 55 60

Met Leu Phe Ala Leu Asp Arg Ile Asn Arg Asp Pro His Leu Leu Pro  
65 70 75 80

Gly Val Arg Leu Gly Ala His Ile Leu Asp Ser Cys Ser Lys Asp Thr  
85 90 95

His Ala Leu Glu Gln Ala Leu Asp Phe Val Arg Ala Ser Leu Ser Arg  
100 105 110

Gly Ala Asp Gly Ser Arg His Ile Cys Pro Asp Gly Ser Tyr Ala Thr  
115 120 125

His Gly Asp Ala Pro Thr Ala Ile Thr Gly Val Ile Gly Gly Ser Tyr  
130 135 140

Ser Asp Val Ser Ile Gln Val Ala Asn Leu Leu Arg Leu Phe Gln Ile  
145 150 155 160

Pro Gln Ile Ser Tyr Ala Ser Thr Ser Ala Lys Leu Ser Asp Lys Ser  
165 170 175

Arg Tyr Asp Tyr Phe Ala Arg Thr Val Pro Pro Asp Phe Phe Gln Ala  
180 185 190

Lys Ala Met Ala Glu Ile Leu Arg Phe Phe Asn Trp Thr Tyr Val Ser  
195 200 205

Thr Glu Ala Ser Glu Gly Asp Tyr Gly Glu Thr Gly Ile Glu Ala Phe  
210 215 220

Glu Leu Glu Ala Arg Ala Arg Asn Ile Cys Val Ala Thr Ser Glu Lys  
225 230 235 240



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Val Gly Arg Ala Met Ser Arg Ala Ala Phe Glu Gly Val Val Arg Ala  
245 250 255

Leu Leu Gln Lys Pro Ser Ala Arg Val Ala Val Leu Phe Thr Arg Ser  
260 265 270

Glu Asp Ala Arg Glu Leu Leu Ala Ala Ser Gln Arg Leu Asn Ala Ser  
275 280 285

Phe Thr Trp Val Ala Ser Asp Gly Trp Gly Ala Leu Glu Ser Val Val  
290 295 300

Ala Gly Ser Glu Gly Ala Ala Glu Gly Ala Ile Thr Ile Glu Leu Ala  
305 310 315 320

Ser Tyr Pro Ile Ser Asp Phe Ala Ser Tyr Phe Gln Ser Leu Asp Pro  
325 330 335

Trp Asn Asn Ser Arg Asn Pro Trp Phe Arg Glu Phe Trp Glu Gln Arg  
340 345 350

Phe Arg Cys Ser Phe Arg Gln Arg Asp Cys Ala Ala His Ser Leu Arg  
355 360 365

Ala Val Pro Phe Glu Gln Glu Ser Lys Ile Met Phe Val Val Asn Ala  
370 375 380

Val Tyr Ala Met Ala His Ala Leu His Asn Met His Arg Ala Leu Cys  
385 390 395 400

Pro Asn Thr Thr Arg Leu Cys Asp Ala Met Arg Pro Val Asn Gly Arg  
405 410 415

Arg Leu Tyr Lys Asp Phe Val Leu Asn Val Lys Phe Asp Ala Pro Phe  
420 425 430

- 40 -

Arg Pro Ala Asp Thr His Asn Glu Val Arg Phe Asp Arg Phe Gly Asp  
435 440 445

Gly Ile Gly Arg Tyr Asn Ile Phe Thr Tyr Leu Arg Ala Gly Ser Gly  
450 455 460

Arg Tyr Arg Tyr Gln Lys Val Gly Tyr Trp Ala Glu Gly Leu Thr Leu  
465 470 475 480

Asp Thr Ser Leu Ile Pro Trp Ala Ser Pro Ser Ala Gly Pro Leu Ala  
485 490 495

Ala Ser Arg Cys Ser Glu Pro Cys Leu Gln Asn Glu Val Lys Ser Val  
500 505 510

Gln Pro Gly Glu Val Cys Cys Trp Leu Cys Ile Pro Cys Gln Pro Tyr  
515 520 525

Glu Tyr Arg Leu Asp Glu Phe Thr Cys Ala Asp Cys Gly Leu Gly Tyr  
530 535 540

Trp Pro Asn Ala Ser Leu Thr Gly Cys Phe Glu Leu Pro Gln Glu Tyr  
545 550 555 560

Ile Arg Trp Gly Asp Ala Trp Ala Val Gly Pro Val Thr Ile Ala Cys  
565 570 575

Leu Gly Ala Leu Ala Thr Leu Phe Val Leu Gly Val Phe Val Arg His  
580 585 590

Asn Ala Thr Pro Val Val Lys Ala Ser Gly Arg Glu Leu Cys Tyr Ile  
595 600 605

Leu Leu Gly Gly Val Phe Leu Cys Tyr Cys Met Thr Phe Ile Phe Ile  
610 615 620

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Lys | Pro | Ser | Thr | Ala | Val | Cys | Thr | Leu | Arg | Arg | Leu | Gly | Leu | Gly | 625 | 630 | 635 | 640 |
| Thr | Ala | Phe | Ser | Val | Cys | Tyr | Ser | Ala | Leu | Leu | Thr | Lys | Thr | Asn | Arg | 645 | 650 | 655 |     |
| Ile | Ala | Arg | Ile | Phe | Gly | Gly | Ala | Arg | Glu | Gly | Ala | Gln | Arg | Pro | Arg | 660 | 665 | 670 |     |
| Phe | Ile | Ser | Pro | Ala | Ser | Gln | Val | Ala | Ile | Cys | Leu | Ala | Leu | Ile | Ser | 675 | 680 | 685 |     |
| Gly | Gln | Leu | Leu | Ile | Val | Val | Ala | Trp | Leu | Val | Val | Glu | Ala | Pro | Gly | 690 | 695 | 700 |     |
| Thr | Gly | Lys | Glu | Thr | Ala | Pro | Glu | Arg | Arg | Glu | Val | Val | Thr | Leu | Arg | 705 | 710 | 715 | 720 |
| Cys | Asn | His | Arg | Asp | Ala | Ser | Met | Leu | Gly | Ser | Leu | Ala | Tyr | Asn | Val | 725 | 730 | 735 |     |
| Leu | Leu | Ile | Ala | Leu | Cys | Thr | Leu | Tyr | Ala | Phe | Asn | Thr | Arg | Lys | Cys | 740 | 745 | 750 |     |
| Pro | Glu | Asn | Phe | Asn | Glu | Ala | Lys | Phe | Ile | Gly | Phe | Thr | Met | Tyr | Thr | 755 | 760 | 765 |     |
| Thr | Cys | Ile | Ile | Trp | Leu | Ala | Leu | Leu | Pro | Ile | Phe | Tyr | Val | Thr | Ser | 770 | 775 | 780 |     |
| Ser | Asp | Tyr | Arg | Val | Gln | Thr | Thr | Thr | Met | Cys | Val | Ser | Val | Ser | Leu | 785 | 790 | 795 | 800 |
| Ser | Gly | Ser | Val | Val | Leu | Gly | Cys | Leu | Phe | Ala | Pro | Lys | Leu | His | Ile | 805 | 810 | 815 |     |

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Ile Leu Phe Gln Pro Gln Lys Asn Val Val Ser His Arg Ala Pro Thr  
820 825 830

Ser Arg Phe Gly Ser Ala Ala Ala Arg Ala Ser Ser Ser Leu Gly Gln  
835 840 845

Gly Ser Gly Ser Gln Phe Val Pro Thr Val Cys Asn Gly Arg Glu Val  
850 855 860

Val Asp Ser Thr Thr Ser Ser Leu  
865 870

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### Claims

1. Purified human metabotropic glutamate receptor (hmGluR) 2.
2. A receptor according to claim 1 which has the amino acid sequence set forth in SEQ ID NO:2.
3. A variant of the receptor of claim 1 or claim 2.
4. Composition of matter comprising a receptor of claim 1.
5. Process for the preparation of a receptor of claim 1 comprising multiplication of a suitable host cell in vitro or in vivo.
6. Use of a receptor according to any of claims 1 to 3 for the screening for a compound which modulates the activity of said receptor.
7. A fusion protein comprising a receptor according to any of claims 1 to 3.
8. Nucleic acid comprising a nucleic acid coding for a receptor according to any of claims 1 to 3, or a fragment of said nucleic acid.
9. Nucleic acid according to claim 8, which is a DNA.
10. A DNA according to claim 9 having the nucleotide sequence set forth in SEQ ID NO:1.
11. Nucleic acid probe comprising at least 14 contiguous bases of the DNA according to claim 9 or 10, or the complement thereof.
12. Process for the preparation of a nucleic acid according to claim 11.
13. A DNA according to claim 9 which is a hybrid vector.
14. A host cell comprising a DNA of claim 9.

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15. A eukaryotic host cell according to claim 14 expressing a DNA coding for a protein according to claim 1.
16. A host cell transfected with a DNA of claim 9.
17. A host cell according to claim 16 which is a mammalian cell.
18. Use of a host cell according to claim 16 for the screening of a compound which modulates the activity of a receptor according to claim 1.
19. Process for the preparation of a host cell according to claim 14.
20. Purified mRNA complementary to the DNA according to claim 9.
21. A method for identifying DNA encoding the hmGluR subtype according to claim 1 comprising: contacting human DNA with a probe according to claim 11, and identifying DNA(s) which substantially hybridize to said probe.
22. A method for identifying compounds binding to hmGluR2 comprising use of the receptor protein according to claim 1 in a competitive binding assay.
23. An assay for identifying compounds which modulate the activity of the hmGluR according to claim 1 comprising
  - contacting the cells of claim 15 with at least one compound or signal whose ability to modulate the activity of said receptor is sought to be determined, and subsequently
  - analyzing cells for a difference in functional response mediated by said receptor.
24. Assay according to claim 23 comprising
  - contacting the cells of claim 15 with at least one compound or signal whose ability to modulate activity of hmGluR2 is sought to be determined, and subsequently
  - monitoring said cells for a change in the level of a particular second messenger.
25. A method for modulating the signal transduction activity of the hmGluR subtype according to claim 1 comprising contacting said subtype with an effective amount of at least one compound identified in the assay of claim 23.

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26. An agonist, antagonist or allosteric modulator identified by the assay of claim 23.
27. A method for detecting a glutamate agonist or an allosteric modulator of hmGluR2 having agonistic activity comprising the steps of (a) exposing a compound to the hmGluR of claim 1 coupled to a response pathway, under conditions and for a time sufficient to allow interaction of the compound with the receptor and an associated response through the pathway, and (b) detecting an increase or decrease in the stimulation of the response pathway resulting from the interaction of the compound with hmGluR2, relative to the absence of the tested compound and therefrom determining the presence of an agonist or an allosteric modulator.
28. A method for identifying a glutamate antagonist or an allosteric modulator of hmGluR2 having antagonistic activity, said method comprising the steps of (a) exposing a compound in the presence of a known glutamate agonist to the hmGluR according to claim 1 coupled to a response pathway, under conditions and for a time sufficient to allow interaction of the agonist with the receptor and an associated response through the pathway, and (b) detecting an inhibition of the stimulation of the response pathway by the agonist resulting from the interaction of the test compound with hmGluR2, relative to the stimulation of the response pathway induced by the glutamate agonist alone, and therefrom determining the presence of an antagonist or an allosteric modulator having antagonist-like activity.
29. An antibody directed against the protein of claim 1.
30. An antibody according to claim 29 which is a polyclonal antibody.
31. An antibody according to claim 29 which is a monoclonal antibody.
32. A method for modulating the signal transduction activity of the hmGluR subtype according to claim 1 comprising contacting said receptor with an antibody of claim 29.
33. A receptor according to claim 1 obtainable by recombinant DNA technology.
34. A transgenic non-human animal which does not express an endogenous mGluR2 gene but a nucleic acid encoding the receptor according to any of claims 1 to 3.

## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 95/02728

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C12N15/85 C07K14/705 C12Q1/00  
 C12N5/10 C12Q1/68 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N C12Q A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.     |
|------------|---|---------------------------|
| X          | NEURON,<br>vol. 8, no. 1, January 1992<br>pages 169-179,<br>YASUTO TANABE ET AL. 'A family of<br>metabotropic glutamate receptors'<br>cited in the application  | 1-6,<br>8-10,<br>13-20,33 |
| Y          | see abstract<br>see page 169, right column, paragraph 2 -<br>page 171, right column, paragraph 1<br>see page 174, right column, last paragraph<br>- page 175, right column, paragraph 2<br>see page 177, right column, paragraph 1 -<br>page 178, left column, paragraph 2<br>---<br>-/-- | 7,11,12,<br>21-28         |

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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\*&\* document member of the same patent family

Date of the actual completion of the international search

21 November 1995

Date of mailing of the international search report

05.12.95

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Authorized officer

Montero Lopez, B



# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 95/02728

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |  |                       |
|--|--|-----------------------|
| Category   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
| Y  | EP,A,0 569 240 (ELI LILLY AND COMPANY) 10<br>November 1993<br>see page 2, line 1 - line 2<br>see page 2, line 21 - line 25<br>see page 2, line 30 - line 39<br>see page 2, line 47 - page 3, line 26<br>see page 12, line 19 - line 33<br>---  | 7,11,12,<br>21-28     |
| P,X  | WO,A,94 29449 (THE SALK INSTITUTE<br>BIOTECHNOLOGY/INDUSTRIAL ASSOCIATES, INC.)<br>22 December 1994<br>see page 3, line 5 - line 24<br>see page 4, line 14 - line 19<br>see page 5, line 20 - line 24<br>see page 10, line 32 - page 11, line 6<br>see page 12, line 1 - line 14<br>see page 13, line 19 - page 14, line 20<br>see page 14, line 33 - page 17, line 6<br>see page 17, line 18 - line 24<br>see page 25, line 4 - line 35<br>see page 28, line 21 - page 30, line 5<br>see page 30, line 21 - page 31, line 19<br>see page 32, line 18 - page 36, line 7<br>see page 52, line 13 - line 30<br>--- | 1,3-9,<br>11-33       |
| P,X  | EUROPEAN JOURNAL OF NEUROSCIENCE,<br>vol. 7, no. 4, 1 April 1995<br>pages 622-629,<br>P.J. FLOR ET AL. 'Molecular cloning,<br>functional expression and pharmacological<br>characterization of the human metabotropic<br>glutamate receptor type 2'<br>see abstract<br>see page 623, right column, paragraph 4 -<br>page 624, right column, paragraph 1;<br>figure 1<br>see page 625, left column, paragraph 1 -<br>page 626, left column, paragraph 1<br>see page 626, left column, paragraph 3 -<br>page 627, left column, paragraph 1<br>-----  | 1-6,<br>8-30,33       |

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 95/02728

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| EP-A-569240                               | 10-11-93            | JP-A- 6022787              | 01-02-94            |
| -----                                     |                     |                            |                     |
| WO-A-9429449                              | 22-12-94            | AU-B- 7098994              | 03-01-95            |
|   |                     | GB-A- 2286398              | 16-08-95            |
| -----                                     |                     |                            |                     |